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CHARACTERIZATION OF SOIL-ROOT INTERACTIONS USING MEDICAL X-RAY COMPUTED TOMOGRAPHY TECHNIQUE

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ABSTRACT

Soil-root interactions in relation to environmental effects and crop productivity are important in agricultural science. The objective of this study aims at investigating a medical X-ray CT (X-ray computed tomography) scanning technique for studying the soil-root interactions and assesses its limitations. A pot experiment was carried out using compost and Ammonium sulfate compost tea (ACT) with and/or without chemical nitrogen fertilizer on growth of radish (*Raphanussativus*) in a sandy soil following root growth using X-ray CT images combined with the ImageJ software. Soil amended with recommended dose of chemical compost plus the recommended dose of chemical nitrogen (RDC+RDN) gave the most efficient treatment that the NPK-uptake of root and shoot was the highest value compared to the other treatment. The obtained images clearly visualized the architecture and morphology of root into the soil matrix. Results of plant growth parameters and NPK-uptake were closely related to the morphological parameters measured using the medical X-ray CT technique. The technique made it possible to isolate the root from the soil and partially overcome the problem of partial volume effect. These technologies may help to elucidate how roots respond to below-ground abiotic stresses in terms of interactions with the biotic environment. The populations of bacteria increased with increasing the soil content of compost and compost tea and the carbon energy sources leaked from roots stimulates the number of heterotrophic bacteria and free-living N_2-fixers.This approach enabled observing root growth manner and how individual roots overcome obstacles on their way and increase understanding of soil plant relationship.

KEYWORDS: Medical X-ray Computed Tomography; Soil-Plant Interactions; Root Morphology; Root Architecture.

INTRODUCTION

Roots have an extreme impact on soil properties, and understanding their development and interactions with soil parameters are essential toward successful crop management (Anon, 2010). Roots embody nonvisible part of plant biology in which is involved (Waisel et al., 2002). They are represented in the activity of microorganisms and hence soil organic matter decomposition (Gregory, 2006). Several methodologies have been used to investigate root developments. Conventional techniques include semi-transparent nutrient agar growth media (Clark et al., 1999; French et al., 2009) and/or gellan (Clark et al., 2011) as artificial growth media. Hence the root washing method is the most common technique to study plant root systems in soil (Smit et al., 2000; Gregory, 2006). Spatial distribution of roots is lost using this method which involves breakage of fine roots. Rhizotron and minirhizotron techniques have extensively been used (Vamerali et al., 1999; Johnson et al., 2001) in roots grown in soil, to identify the direction of root growth. However, observations indicate

only small fractions of the entire root system, and are limited to the boundary surface. An alternative technique is X-ray computed tomography (CT), a non-destructive quantitative technique that can visualize and image opaque objects. It provides new parameter insights of soil-root-water interactions (Pierret et al., 2005; Jahnke et al., 2009). This technique has been intensively used to show the soil morphology in three-dimensional set-up without soil destruction (Anderson et al., 1990; Naveed et al., 2013). For identifying soil architecture and functions, Naveed et al. (2013) investigated linking X-ray CT measurements on soil pore parameters with the conventional soil physical measurements. Image analysis using X-ray CT scanners involve the use of medical CT from 500 mm to 200 nm along with nano-CT scanners to quantify three dimensional structures of soil pores and their connectivity. Cnudde et al. (2006) showed that images with medical CT facilitate scanning of larger (10–100 cm) samples, and its higher spatial resolution is limited to smaller (0.1–1 cm) samples. Sander et al. (2008) assessed paddy soil structure in soil columns using medical CT with spatial resolution of 0.25 mm horizontally and 1 mm vertically. For the three dimensional visualization and quantification in soil science, Taina et al. (2008) consider the X-ray CT scanning technique is a unique tool, where its spatial resolution ranges from 10–500 μm depending on the scanner type and sample size. A CT scanner requires several projections for the different angles used for reconstruction of the CT data in three-dimensional systems. For visualization purposes, Mooney (2002) reported that the data are mapped to grayscale intensity values and are expressed in Hounsfield units. Gregory et al. (2006), Jenneson et al. (2003), and Tracy et al. (2010) showed that CT is an efficient tool to visualize plant root system. The overlap in x-ray attenuation values of organic matter and plant roots in soil was the limiting obstacle, where their variations are caused by water stored in soil pores and/or retained in roots. Heeraman et al. (1997) reported that root extractions were difficult using current based image analysis thresholding approaches for quantification the root material using a per-voxel basis. These voxels were defined to different classes reflecting the scanned specimen components, and building a single model expected from materials of X-ray attenuation data. This approach was conscious to noise and required clear distinction between the grayscale values of the object and its background values (Pierret et al., 1999; Lontoc-Roy et al., 2006; Kaestner et al., 2006; Perret et al., 2007). Although the thresholding techniques alone scarcely supplies sufficient accurate results, it is often combined with additional operations. Pierret et al. (1999) and Kaestner et al. (2006) preformed several morphological operations of post-processing for determining the remaining voxels that could belong to the final outcome or not. Lontoc-Roy et al. (2006) and Perret et al. (2007) provide an explicit connectivity check methods to coincide voxels in the threshold limits and not part of the roots. The objective of the present work was to investigate and modify an easy protocol/method to identify and describe the tape/main root morphology growing using a medical CT scanning technique and assess the potentials and limitations of this technique.

MATERAIL AND METHOD

Quantification of Root Architecture Using Medical CT

Imaging with a CT scanner is based on measurements of the different attenuation of X-rays passing through various kinds of matter of the sample. Image acquisition was done at El-Nour Radiology Centre (Egypt) with an X-ray CT of Siemens ECLOS (Medical Corporation). Soil pots with their plants were placed on the bench and scanned by the X-ray medical scanner (Figure 1).

Figure 1. Detector X-ray source in rotation around the sample, it generates a 3D image from a series of 2D images taken around a single axis of rotation as a non-destructive method.

The scanner consisted of a gantry with a patient couch for positioning and advancing of the patient. The data were reconstructed on a three-dimensional matrix with the voxel values directly reflecting the linear attenuation coefficient in the field of view. The values are reported in Hounsfield units (HU) (Hounsfield, 1973), where attenuation of air and water are set to −1,000 and 0 respectively. After scanning and reconstruction, 256 slices were produced, in coronal view. The CT images (16 bit) had a voxel dimension of 0.39 × 0.39 × 0.6 mm for all the 96 image stacks (corresponding to the 32 samples scanned three times).

Image Processing and Analysis

Different steps were required for root structure analysis. The analyses were made using the software ImageJ (Rasband, 2009). The images were selected and segmented using the automatic Otsu thresholding algorithm provided in the ImageJ software (version 1.45K) to isolate the root from the soil. This results in binary images from the original grey-level images. Subsequent image analysis was carried out using the binary images. The segmentation method used to isolate the root system was based on a thresholding method similar to the method described by Lontoc-Roy et al. (2006).

Selected Region of Interest (ROI)

The identical ROIs from the three sets of images were selected and segmented using a thresholding method producing binary images from the grey-level ones. Segmentation of the images was carried out to discriminate the main root (the object) from the soil matrix (the background). Image analysis was carried out using both the binary images and the original grey scale images, calibrated in HU units.

Main Root Morphological Parameters

Marching cubes algorithm, implemented in an ImageJ plugin, BoneJ, was used to render iso-surface on 3D volumetric data to calculate surface area and volume of roots (Lorensen and Cline, 1987). Surface area of the roots (AS) and its volume (AV) were calculated using this algorithm. With images of the binaries, the main root thickness could be defined as the diameter of the most fitting sphere with centre P (x, y, z) onto the root structure. The plug in local thickness (Dougherty and Kunzelmann, 2007) gave the mean thickness of the main root and the thickness map is displayed at each point P of the root. The 3D coordinates X, Y, and Z of the geometrical centre for each root were identified. A thinning algorithm was applied to reduce iteratively the diameter of root until only a skeleton remained (Lee et al., 1994) and available as a plug-in, 3D skeletonize, in ImageJ. Besides, the ImageJ plugin was used and skeleton analysis was done to analyses these networks. Therefore characterization of the largest shortest path for each network was attained. The largest

shortest path is an estimate of the Euclidian distance obtained with the Floyd-Warshall Algorithm.

Plant Material and Growth Conditions

A pot experiment was carried out to examine the effect of using compost and a modified compost tea (Ammonium sulfate compost tea) combined with chemical nitrogen fertilizers on growth of radish (*Raphanussativus*). Medical X-ray CT technique was used to study the interaction of soil with roots of the plant. Soil garbage compost "C" having organic carbon content of 30%, total nitrogen 1.0%, moisture 35%, and pH 7.5 was used. Ammonium sulfate compost tea (ACT) was prepared by the modification of the method of Ingham (2005) with adding 1 g L^{-1} of $(NH_4)_2$ SO_4. The water used through the compost preparation was pump aerated for 30 min. to remove chlorine. Compost soaking was done at room temperature for 96 hr., while being aerated continuously (10 L/min air delivery per bucket through air stones). Its properties were as follows: EC 2.3 dSm^{-1}, pH 7.1, total C and N being 6.2 and 3.4 g L^{-1}, respectively. The soil properties were as follows: EC (past extract) 0.51 dSm^{-1}, pH 8.02, field capacity 9.14%, organic matter 4.8 g kg^{-1}, and $CaCO_3$ 5.0 g kg^{-1}. Analysis was prepared according to methods cited by AOAC, 2002 and Baruah & Barthakur (1997). The soil was prepared by packing 6 kg pot^{-1} (Ø=20 cm, height=25 cm) and set in a vertical position. Plant seeds were sown on the 5^{th} of November 2013. The experimental design was a randomized complete block with three replicates. Plants were thinned to one plant per pot after 15 days of sowing. There were ten treatments as follows: control, recommended dose of N–fertilizers "RDN" (240 kg N ha^{-1}) as ammonium sulphate, recommended dose of compost "RDC" (24 ton. ha^{-1}), RDN + RDC, Ammonium sulfate compost tea "ACT" + RDC, ACT "1:25" (i.e. diluted by water v/v) + RDN, ACT "1:50" + RDN, ACT "1:75" + RDN, ACT "1:25" + 1/2 RDN, ACT "1:50" + 1/2 RDN, and ACT "1:75" + 1/2 RDN. To assess the impact of different treatment on root behavior and the surrounded soil matrix, N, P, and K uptake of the root and shoot were measured according to Cottenie et al. (1982). In addition, soil microorganisms was characterized, that the microbial populations were undertaken directly in soil samples collected at 0, 4, and 8 weeks of sowing, to determine soil biota (total bacteria, aerobic N_2-fixing bacteria, actinomycetes, and fungi). Eight weeks after sowing, growth parameters of number of leaves/ plant, root fresh and dry weight, shoot fresh and dry weight were recorded.

RESULTS

Plant Parameters

Table 1 shows the growth parameters of radish under different treatment as number of leaves per plant, root fresh and dry weight, shoots fresh and dry weight. Soil amended with RDC + RDN gave the highest fresh and dry weight of roots (31.80 g $plant^{-1}$ and 3.47 g $plant^{-1}$, respectively) as well as the highest fresh and dry weight of shoots (54.10 g $plant^{-1}$ and 6.58 g $plant^{-1}$, respectively). While the lowest values of fresh and dry weight of root were noticed in the control treatment (8.67 g $plant^{-1}$ and 0.49 g $plant^{-1}$, respectively) as well as the lowest fresh and dry weight of shoots (9.17 g $plant^{-1}$ and 0.86 g $plant^{-1}$, respectively).

Table 1: Effect of Compost and Compost Extract Combined with Chemical N- Fertilizers on Growth Parameters of Radish Plants under Pot Conditions

Treatment	Leaves/ Plant	Root F.W (g/Plant)	Root D.W (g/Plant)	Shoot F.W (g/Plant)	Shoot D.W (g/Plant)
Control	9.00	8.67 c	0.49 c	9.17 e	0.86 e
RDN	14.00	31.77 a	2.47 ab	46.17 a	3.57 b
RDC	11.33	24.67 ab	1.63 b	27.73 c	2.40 cd
Table 1- Cont.,					
RDC + RDN	12.67	31.80 a	3.47 a	54.10 a	6.58 a

ACT (1:25) + RDN	13.67	28.8 a	3.41 a	34.73 b	3.61 b
ACT (1:50) + RDN	12.00	21.20 b	3.34 a	38.70 b	3.98 b
ACT (1:75) + RDN	12.67	25.77 ab	4.27 a	35.93 b	4.45 ab
ACT (1:25) + 1/2 RDN	11.67	24.23 ab	3.69 a	32.13 bc	2.92 d
ACT (1:50) +1/2 RDN	11.33	22.47 ab	3.93 a	33.93 b	2.36 d
ACT (1:75) + 1/2 RDN	10.33	19.96 bc	3.46 a	27.8 c	2.73 d

Numbers in the same column with different letters are significantly different ($P \leq 0.05$). RDN: recommended dose of chemical N–fertilizers; RDC: recommended dose of compost; RDC +RDN; ACT-25 + RDN: Soil amended with Ammonium sulfate compost tea ACT (1:25 v/v water) + RDN; ACT-50 + RDN: Soil amended with ACT (1:50 v/v water) + RDN; ACT-75 + RDN; Soil amended with ACT (1:75 v/v water) + RDN.

Table 2 shows the N, P and K uptake in root and shoot of radish under the different treatments. Soil amended with RDC+RDN gave the most efficient treatment that the NPK-uptake of root and shoot was the highest value compared to the other treatments. That RDC+RDN gave the highest NPK-uptake of shoots (83.40 mg pot^{-1}, 29.50 mg pot^{-1}, and 180.10mg pot^{-1}, respectively) as well as the highest NPK-uptake of roots (45.20 mg pot^{-1}, 11.60 mg pot^{-1}, and 107.31 mg pot^{-1}, respectively). The results of growth parameters (Table 1) and the NPK-uptake (Table 2) of root and shoot were closely related to the morphological parameters measured by X-ray CT techniques (Table 3). That the treatment (RDC + RDN) gave the highest values of longest root depth, root volume, surface, thickness, and tortuosity.

Table 2: Influence of Compost and Compost Tea on N, P and K Uptake in Root and Shoot of Radish

Treatment	Shoot Nutrient Uptake			Root Nutrient Uptake		
	N Uptake (mg Pot^{-1})	P Uptake (mg Pot^{-1})	K Uptake (mg Pot^{-1})	N Uptake (mg Pot^{-1})	P Uptake (mg Pot^{-1})	K Uptake (mg Pot^{-1})
Control	33.41 d	15.35 c	50.95 d	18.76 d	5.31 c	40.10 c
RDN	68.10 b	22.65 b	98.67 c	38.52 b	8.30 b	73.62 b
RDC	41.25 c	16.22 c	128.60 b	30.60 c	8.90 b	81.59 b
RDC + RDN	83.40 a	29.50 a	180.10 a	45.20 a	11.60 a	107.31 a
ACT (1:25) + RDN	74.37 a	18.76 bc	67.74 cd	39.15 b	5.40 c	56.30 c
ACT (1:50) + RDN	68.57 b	21.50 b	101.01 bc	38.35 b	9.02 b	56.09 c
ACT (1:75) + RDN	66.14 b	23.58 b	88.76 c	37.78 b	8.08 bc	57.16 c
ACT (1:25) + 1/2 RDN	50.17 bc	16.41 c	59.11 d	27.01 c	7.88 c	55.52 c
ACT (1:50) +1/2 RDN	49.87 bc	20.90 bc	55.01 d	39.57 b	6.37 c	54.79 c
ACT (1:75) + 1/2 RDN	43.93 c	20.02 bc	61.94 d	28.80 c	6.65 c	50.60 c

Numbers in the same column with different letters are significantly different ($P \leq 0.05$). RDN: recommended dose of chemical N–fertilizers; RDC: recommended dose of compost; RDC +RDN; ACT-25 + RDN: Soil amended with Ammonium sulfate compost tea ACT (1:25 v/v water) + RDN; ACT-50 + RDN: Soil amended with ACT (1:50 v/v water) + RDN; ACT-75 + RDN; Soil amended with ACT (1:75 v/v water) + RDN.

Root Morphological Characteristics

Root morphological characteristics varied among treatments and have major effects on total root parameters and their 3-D architecture (Table 3). Main roots were traced and measured by following the shape of the root path, thereby providing accurate measurements of the root length as it extends down the soil pots (Table 3). Results of X-ray computed tomography reveal that soil amended with RDC+RDN gave the longest root depth of 149.3 mm, while the lowest root depth 56.9 mm was found the control treatment (Table 3). The same treatment (RDC+RDN) showed highest morphological root parameters of volume, surface, thickness, and tortuosity.

Table3: Root morphological Parameters Measured by Medical X-Ray CT of Different Treatments

	Root Morphometric Parameters									
Sample ID	Volume (mm^3)	Mean thickness (mm)	Max. thickness (mm)	Surface (mm^2)	Longest shortest path (mm)	Tortuosity	Coordinate of center of mass (mm) X	Y	Z	Depth (mm)
C	1377.8	3.0	4.5	1919.4	71.3	0.8	14.3	30.1	6.4	56.9
RDN	12288.0	7.4	11.1	7646.4	169.6	0.8	16.6	52.1	10.6	139.7
RDC	7108.9	6.2	10.5	6432.2	169.8	0.5	30.0	25.6	20.5	93.2
RDC + RDN	13072.0	7.0	11.7	10155.1	188.2	0.8	22.6	82.6	9.4	149.3
CT1+RDN	1709.3	2.5	4.3	3373.0	150.0	0.6	18.0	49.3	16.6	83.6
CT2+RDN	8497.2	4.8	9.1	9236.3	218.0	0.7	43.6	77.8	23.7	140.0
CT3+RDN	4957.3	4.6	8.3	5747.7	183.2	0.7	11.9	52.7	9.9	120.6
CT4+1/2RDN	8365.8	5.3	8.5	7903.5	161.9	0.7	22.9	43.3	11.0	106.2
CT5+1/2RDN	6089.1	4.3	7.4	6401.3	180.6	0.6	14.5	29.9	23.8	113.7

C: Control (without any additives); RDN: recommended dose of chemical N-fertilizers; RDC: recommended dose of compost; CT1+ RDN: Soil amended with Ammonium sulfate compost tea ACT (1:25 v/v water) + RDN; CT2 + RDN: Soil amended with ACT (1:50 v/v water) + RDN; CT3 + RDN: Soil amended with ACT (1:75 v/v water) + RDN; CT4 +1/2 RDN: Soil amended with ACT (1:25 v/v water) + 1/2 RDN; CT5 +1/2 RDN: Soil amended with ACT (1:50 v/v water) + 1/2 RDN.

Root Architecture Using Medical CT

Results of the isolated main root system are shown in Figures 2. The obtained results were prepared whilst roots are still encased within the soil matrix. Figure 2 shows 3D visualization of the X-ray CT detected root of soil amended with RDN. The left image shows the pore thickness in 3D and 2D with the calibration thickness in mm. This level of details of growth medium structure could not be achieved using traditional methods for root studies, but using x-ray computed tomography enables following the root growth into the soil. The treatment (RDC+RDN) showed highest morphological root parameters as max thickness that close of the soil surface compared to the other treatments.

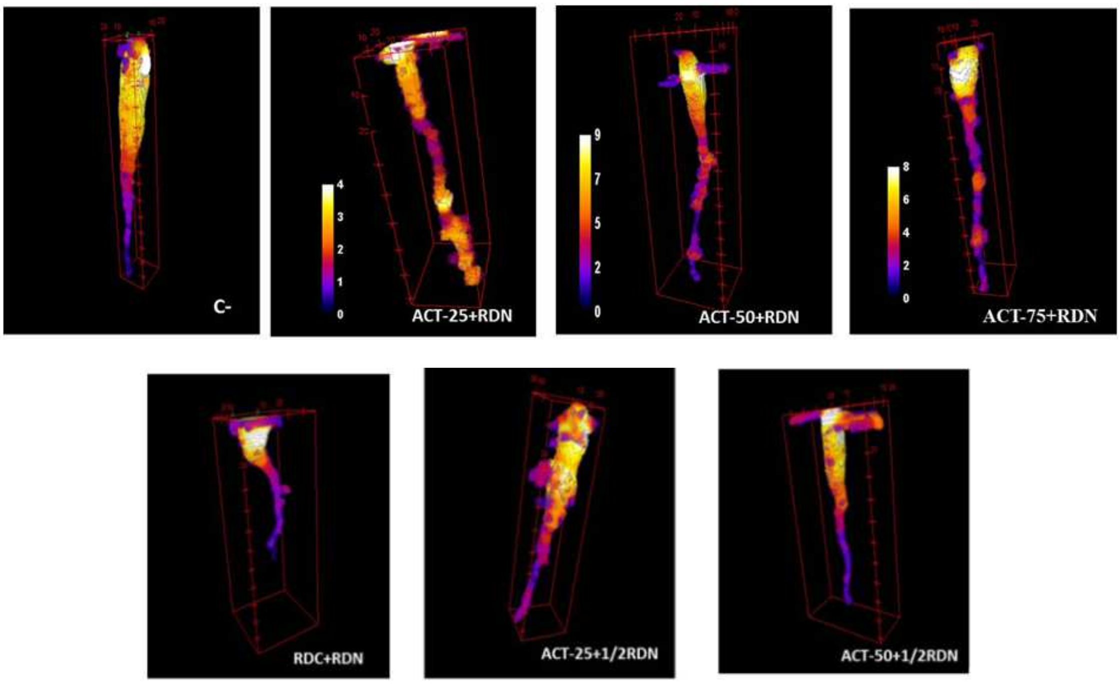

Figure 2. 3D Visualization of the Main Root Into the Soil Matrix.The Images Show the Pore Thickness in 3D with Their Respective Calibration Bar in mm for Root of Soil Amended with ACT-25+RDN, ACT-50+RDN, ACT-75+RDN, RDC+RDN, ACT-25+1/2RDN, ACT-50+1/2RDN Compared to the Control Treatment (C-).

With the binary images, the main root thickness distribution versus the root depth expressed as the diameter of the best fitted sphere with centre P (x, y, z) onto the root structure is shown in figure 3. Using this process, the skeleton lines in a medial position was performed symmetrically and kept the connectedness of the root volume. Results of figure 3 show that for all curves root thickness increase within the first mm of the root. It is followed then by decrease of thickness. RDC shows a maximum root thickness at 10 voxels depth (i.e. 4 mm). Using this technique, the highest root thickness was identified for the RDC+ RDN treatment that noticed at 30voxels depth (i.e. 12 mm) compared the other treatments.

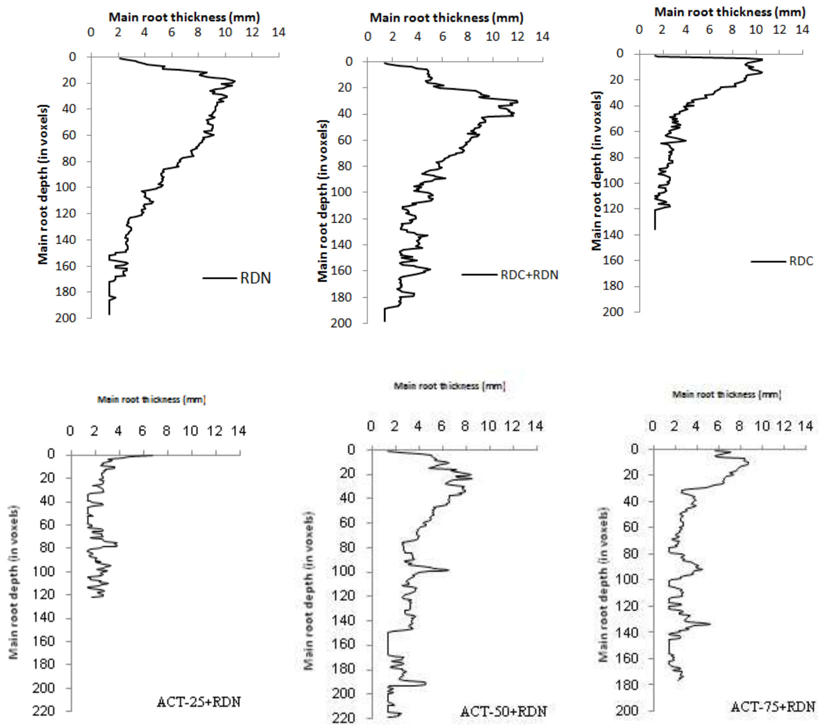

Figure 3. Characteristics Main Root Thickness (mm) Versus Root Depth (voxels) Using X-ray CT Data Under Different Treatments; RDN: Recommended Dose of Chemical N–fertilizers; RDC: Recommended Dose of Compost; RDC +RDN; Act-25 + RDN: Soil Amended with ACT (1:25 V/V Water) + RDN; Act-50 + RDN: Soil Amended with ACT (1:50 V/V Water) + RDN; ACT-75 + RDN; Soil Amended with ACT (1:75 V/V Water) + RDN.

Soil microbial Characteristics

Table 4 shows the soil microbial population affected by the different treatments. Values increased with increasing the rate of compost and compost tea. Bacterial populations showed significant increase in almost all treatments that contained compost or compost tea compared with the other treatments. Aerobic N-fixing bacteria counts associated with the rhizosphere are presented in Table 4. Counts of aerobic N-fixing bacteria in the latter periods were markedly greater compared with counts of the earlier period. Treatments containing high levels of chemical N-fertilizer, exhibited a significant decrease in aerobic N_2-fixing bacteria populations after 8 weeks of cultivation in contrast with a lesser extent with the 4 weeks period. Treatments containing compost tea reflected lower fungal counts particularly after 8 weeks. As for the fungi, the compost and RDC + RDN were occupied the first rank and had statistically the highest values. While, the other tested treatments came in the second rank without significant differences between them.

DISCUSSIONS

Roots have an extreme impact on soil properties, and understanding their development and interactions with soil parameters are essential toward successful crop management (Anon, 2010). Roots embody nonvisible part of plant biology in which is involved (Waisel et al., 2002), that reflect the amendments into the soil matrix. The results of table 2 show that using the treatment RDC + RDN was the most efficient treatment of NPK-uptake compared to the other treatment. This result could be attributed to the high nitrogen content and the low C/N ratio in RDC compared to the ACT treatments (Abdallah et al., 2013). Where the beneficial effect of compost tea on NPK-uptake attributed to both supply nutrients and their functions (as useful microorganisms increase the time stomata stay open then reducing loss from the leaf surface) that provide chelated microelements and make them easier for plants to absorb and increasing soil aeration and acidity (Ezz El-Din and Hendawy, 2010). In the current study, medical CT scanning was used to provide structural and functional information about root and soil interaction. In fact, this technology gave visible images of the architecture and morphology of root in soil. It was possible by this modification to reduce the duration of the experiment as well as the risk of sample disturbance compared with the conventional techniques (Jahnke structured soils (Tainaet al., 2008). Using the medical CT scanner enables visualizing the main taproot and detecting it to 30 mm depth (fig. 3). With this, it was possible to envisage a study taking place on plant growth and development subjected to stress, and treatment effects on plants with special focus on the root system. Further understanding and experiments on root growth, resource acquisition, and root-shoot communication under different effects of multiple and combined abiotic stresses (e.g. drought, water logging, salinity, nutrient status, soil physical status) are required. These technologies may help to elucidate how roots respond to below-ground abiotic stresses in terms of development, root-shoot communication and interactions with the biotic environment. The populations of bacteria increased with increasing the soil content of compost and compost tea. Carbon energy sources leaked from roots stimulates the number of heterotrophic bacteria and free-living N_2-fixers in the plant experiments (Herman *et al.*, 1993). That is a known fact for the organic matter when it is introduced to soil (Brady and Weil, 1999). This can be explained by the increased nutrition supplement due to the compost and compost tea (Heather *et al.*, 2006).It is noted that the treatments that gave the highest values are not added to it chemical nitrogen or added to lower levels of chemical nitrogen and this corresponds to the fact that increasing nitrogen levels in soil would reduce the number of nitrogen-fixing bacteria in the soil (Streeter, 1988; Herman et al., 1993; Harper, 1997).et al., 2009) to derive the architecture and functional imaging. Using medical CT scanner allowed placing the soil-plant system on the bench where it remained undisturbed during the whole experiment period (i.e. both initial labeling the subsequent scanning phase). The environment was also kept undisturbed during the experiment, with constant temperature and light intensity. With the binary images, it is possible to define the main root thickness as the diameter of the best fitted sphere with centre P (x, y, z) onto the root structure (Table 3). Even the plug in Local thickness (Dougherty and Kunzelmann, 2007) gave the mean thickness of the main root. The 3D coordinates X, Y, and Z of the geometrical centre for each root were identified. By instituting environmental control, it was possible to manipulate the environment and monitor the root-soil response (fig. 2). With a resolution up to 0.6 mm, the CT images were rather similar to the resolution achieved by (Jahnke et al., 2009) using magnetic resonance imaging (MRI) (0.4 mm).

In this study, by using the medical CT scanner, it was possible to extract information on soil and root structure (fig. 2), that previous studies on the structure and functioning of roots were not able to integrate the component structure of the soil (Jahnke et al., 2009). The images of CT fluctuated with depth at higher values at the bottom of the VOI which could be attributed to the distribution of plant material and air surrounding the roots (fig. 3). The CT scanning technique

also allows detailed visualization and quantification of structural properties in differently. Effects of above-ground stresses (e.g., light and temperature) can also be identified. Therefore the medical CT scanner can be used to provide information on root development. Rescanning with a micro-CT scanner may demand termination of plant growth, freezing and transport over long distances. In the present study, for simplicity, homogeneous sand was used as the test soil. This allowed a clear differentiation of roots from sand in CT scanning as emphasized by Lontoc-Roy et al. (2006). Natural soils, with a more complex structure, can be used in future studies using some modifications of the principles described in the current study and other previous studies (Taina et al., 2008).

CONCLUSIONS

X-ray CT is an efficient technique for identifying root-soil interaction offering a major advancement in our understanding the dynamic nature of the soil-plant relationship. The 3D visualization of the X-ray CT detected the root whilst still encased within the soil matrix. Root morphological parameters as well as longest root depth, root volume, surface, thickness, and tortuosity were defined using the binary images. Therefore medical X-ray computed tomography enables following the root growth. Using this technique, the below-ground impact of plant genotype on its phenotype regarding its roots and their soil environment can be visualized. This may help in studying roles of novel genes in optimizing the acquisition of water and nutrients from soil. These technologies help to elucidate how roots respond to below-ground abiotic stresses in terms of development, root-shoot communication and interactions with the biotic environment.

Table 4: Changes in Microbial Populations of Bacteria, Aerobic N_2-Fixing Bacteria Actinomycetes and Fungi in the Soil of Cultivation, During the Time Course of the Experiment

Treatment	Microbial populations (log10 CFU/g [d.w. soil])															
	Bacteria				Aerobic N_2fixing bacteria				Actinomycetes				Fungi			
	sowing time	4 weeks	8 weeks	Av.	Sowing time	4 weeks	8 weeks	Av.	Sowing time	4 weeks	8 weeks	Av.	Sowing time	4 weeks	8 weeks	Av.
Control	6.30b	7.15b	7.35a	6.93b	4.31b	5.80a	6.11a	5.40a	3.44	3.78	4.47	3.90	2.57	3.11	3.21	2.96b
RDN	6.54b	7.8a	7.92a	7.42b	4.28b	3.13c	3.20c	3.54b	3.44	3.81	4.50	3.92	2.57	3.25	3.35	3.06b
RDC	7.90a	8.49a	8.87a	8.42a	4.82a	5.89a	6.15a	5.62a	4.65	4.99	5.68	5.11	3.72	3.98	4.14	3.95a
RDC + RDN	7.93a	8.69a	8.85a	8.49a	4.81a	3.25c	3.30c	3.78b	4.61	4.95	5.60	5.05	3.70	4.02	4.26	3.99a
ACT (1:25) + RDN	7.65a	7.69a	7.80a	7.71a	4.20b	3.10c	3.30c	3.53b	4.19	4.53	5.22	4.65	2.70	2.46	2.59	2.58b
ACT (1:50) + RDN	7.20b	7.65a	7.79a	7.55b	4.13b	3.12c	3.25c	3.50b	4.21	4.55	5.24	4.67	2.85	2.41	2.54	2.60b
ACT (1:75) + RDN	6.71b	7.64a	7.79a	7.38b	4.11b	3.15c	3.34b	3.53b	4.13	4.47	5.16	4.59	2.81	2.40	2.53	2.58b
ACT (1:25) + 1/2 RDN	7.59a	7.59a	7.73a	7.64b	4.20b	4.24b	4.68b	4.37a	4.14	4.48	5.17	4.60	2.91	2.44	2.57	2.64b
ACT (1:50) +1/2 RDN	7.23b	7.59a	7.74a	7.52b	4.31b	4.26b	4.60b	4.39a	4.19	4.53	5.22	4.65	2.83	2.40	2.53	2.59b
ACT(1:75) + 1/2 RDN	6.68b	7.55a	7.73a	7.32b	4.30b	4.26b	4.65b	4.40a	4.15	4.49	5.18	4.61	2.85	2.38	2.51	2.58b
Sampling date av.	7.19b	7.79a	7.96a		4.39a	4.02b	4.23a		4.16b	4.51a	5.19a		2.94	2.65	2.79	
LSD (0.05)	Sampling date	fertilization	Sp × F		Sampling date	fertilization	Sp × F		Sampling date	fertilization	Sp × F		Sampling date	Fertilization	Sp × F	
	0.63	0.05	1.62		0.31	1.30	1.42		0.85	NS	NS		NS	0.78	NS	

RDN: recommended dose of chemical N–fertilizers; RDC: recommended dose of compost; RDC +RDN; ACT-25 + RDN: Soil amended with Ammonium sulfate compost tea ACT (1:25 v/v water) + RDN; ACT-50 + RDN: Soil amended with ACT (1:50 v/v water) + RDN; ACT-75 + RDN; Soil amended with ACT (1:75 v/v water) + RDN.

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